Whole embryo cultures were also utilized by Hunter et al. (1996) in evaluating the embryotoxic potential of a number of disinfection byproducts, including the TCE metabolites DCA and TCA. CD-1 mouse conceptuses (GD 9; 3–6 somites) were cultured for 24–26 hours in treated medium. DCA levels assessed were 0, 734, 1,468, 4,403, 5,871, 7,339, 11,010, or 14,680 μM; TCA levels assessed were 0, 500, 1,000, 2,000, 3,000, 4,000, and 5,000 μM. For DCA, neural tube defects were observed at levels ≥5,871 μM, heart defects were observed at ≥7,339 μM, and eye defects were observed at levels ≥11,010 μM. For TCA, neural tube defects were observed at levels ≥2,000 μM, heart and eye defects were observed at ≥3,000 μM. The heart defects for TCA were reported to include incomplete looping, a reduction in the length of the heart beyond the bulboventricular fold, and a marked reduction in the caliber of the heart tube lumen. Overall benchmark concentrations (i.e., the lower limit of the 95% CI required to produce a 5% increase in the number of embryos with neural tube defects) were 2,451.9 μM for DCA and 1,335.8 μM for TCA (Richard and Hunter, 1996).

Boyer et al. (2000) used an in vitro chick-AVC culture to test the hypothesis that TCE might cause cardiac valve and septal defects by specifically perturbing epithelial-mesenchymal cell transformation of endothelial cells in the AVC and outflow tract areas of the heart. AV explants from Stage 16 White Leghorn chick embryos were placed in hydrated collagen gels, with medium and TCE concentrations of 0, 50, 100, 150, 200, or 250 ppm. TCE was found to block the endothelial cell-cell separation process that is associated with endothelial activation as well as to inhibit mesenchymal cell formation across all TCE concentrations tested. TCE did not, however, have an effect on the cell migration rate of fully formed mesenchymal cells. TCE-treatment was also found to inhibit the expression of transformation factor Mox-1 and extracellular matrix protein fibrillin 2, two protein markers of epithelial-mesenchyme cell transformation.

4.8.3.3. Discussion/Synthesis of Developmental Data

In summary, an overall review of the weight of evidence in humans and experimental animals is suggestive of the potential for developmental toxicity with TCE exposure. A number of developmental outcomes have been observed in the animal toxicity and the epidemiological data, as discussed below. These include adverse fetal/birth outcomes including death (spontaneous abortion, perinatal death, pre- or postimplantation loss, resorptions), decreased growth (low birth weight, SGA, IUGR, decreased postnatal growth), and congenital malformations, in particular cardiac defects. Postnatal developmental outcomes include developmental neurotoxicity, developmental immunotoxicity, and childhood cancer.

4.8.3.3.1. Adverse fetal and early neonatal outcomes

Studies that demonstrate adverse fetal or early neonatal outcomes are summarized in Table 4-102. In human studies of prenatal TCE exposure, increased risk of spontaneous abortion

was observed in some studies (ATSDR, 2001; Taskinen et al., 1994; Windham et al., 1991), but not in others (ATSDR, 2008b, 2001; Goldberg et al., 1990; Lindbohm et al., 1990; Taskinen et al., 1989; Lagakos et al., 1986). In addition, perinatal deaths were observed after 1970, but not before 1970 (Lagakos et al., 1986). In rodent studies that examined offspring viability and survival, there was an indication that TCE exposure may have resulted in increased pre-and/or postimplantation loss (Kumar et al., 2000b; Narotsky and Kavlock, 1995; Healy et al., 1982), and in reductions in live pups born as well as in postnatal and postweaning survival (George et al., 1986; George et al., 1985).

Table 4-102. Summary of adverse fetal and early neonatal outcomes associated with TCE exposures

Positive finding	Species	Reference
Spontaneous abortion, miscarriage, pre-and/or postimplantation loss	Human	ATSDR (2001)[a]; Taskinen et al. (1994)[a]; Windham et al. (1991)
	Rat	Kumar et al. (2000b); Healy et al. (1982); Narotsky and Kavlock (1995); Narotsky et al. (1995)
Perinatal death, reduction in live births	Human	Lagakos et al. (1986)[b]
	Mouse	George et al. (1985)
	Rat	George et al. (1986)
Postnatal and postweaning survival	Mouse	George et al. (1985)
	Rat	George et al. (1986)
Decreased birth weight, SGA, postnatal growth	Human	ATSDR (1998a); ATSDR (2006a); Rodenbeck et al. (2000)[c]; Windham et al. (1991)
	Mouse	George et al. (1985)
	Rat	George et al. (1986); Healy et al. (1982); Narotsky and Kavlock (1995); Narotsky et al. (1995)

[a]Not significant.
[b]Observed for exposures after 1970, but not before.
[c]Increased risk for very low birth weight but not low birth weight or full-term low birth weight.

Decreased birth weight and SGA was observed (ATSDR, 2006a; Rodenbeck et al., 2000; ATSDR, 1998a; Windham et al., 1991); however, no association was observed in other studies (Bove, 1996; Bove et al., 1995; Lagakos et al., 1986). While comprising both occupational and environmental exposures, these human studies are, overall, not highly informative due to their small numbers of cases and limited exposure characterization or to the fact that exposures to mixed solvents were involved. However, decreased fetal weight, live birth weights and postnatal growth were also observed in rodents, (Narotsky and Kavlock, 1995; George et al., 1986; George et al., 1985; Healy et al., 1982), adding to the weight of evidence for this endpoint. It is noted that the rat studies reporting effects on fetal or neonatal viability and growth used F344 or Wistar

rats, while several other studies, which used Sprague-Dawley rats, reported no increased risk in these developmental measures (Carney et al., 2006; Hardin et al., 1981; Schwetz et al., 1975).

Overall, based on weakly suggestive epidemiologic data and fairly consistent laboratory animal data, it can be concluded that TCE exposure poses a potential hazard for prenatal losses and decreased growth or birth weight of offspring.

4.8.3.3.2. Cardiac malformations

A discrete number of epidemiological studies and studies in laboratory animal models have identified an association between TCE exposures and cardiac defects in developing embryos and/or fetuses. These are listed in Table 4-103. Additionally, a number of avian and rodent in vivo studies and in vitro assays have examined various aspects of the induction of cardiac malformations.

Table 4-103. Summary of studies that identified cardiac malformations associated with TCE exposures

Finding	Species	References
Cardiac defects	Human	ATSDR (2008b, 2006a); Yauck et al. (2004)
	Rat	Dawson et al. (1993, 1990); Johnson et al. (2003); Johnson et al. (2005); Johnson et al. (1998b; 1998a)[a]; Smith et al. (1989), (1992)[a]; Epstein et al. (1992)[a]
	Chicken	Bross et al. (1983); Boyer et al. (2000); Loeber et al. (1988); Drake et al. (2006a; 2006b); Mishima et al. (2006); Rufer et al. (2010; 2008)
Altered heart rate	Human	Jasinka (1965, translation)

[a]Metabolites of TCE.

In humans, an increased risk of cardiac defects has been observed after exposure to TCE in studies reported by ATSDR (2008b, 2006a) and Yauck et al. (2004), although others saw no significant effect (Bove, 1996; Bove et al., 1995; Goldberg et al., 1990; Lagakos et al., 1986), possibly due to a small number of cases. In addition, altered heart rate was seen in one study (Jasińska, 1965, translation). A cohort of water contamination in Santa Clara County, California is often cited as a study of TCE exposure and cardiac defects; however, the chemical of exposure is in fact trichloroethane, not TCE (Deane et al., 1989; Swan et al., 1989).

In laboratory animal models, avian studies were the first to identify adverse effects of TCE exposure on cardiac development. As described in Section 4.8.3.2.2.1, cardiac malformations have been reported in chick embryos exposed to TCE (Rufer et al., 2008; Drake et al., 2006a; Drake et al., 2006b; Mishima et al., 2006; Boyer et al., 2000; Loeber et al., 1988; Bross et al., 1983). Additionally, a number of studies were conducted in rodents in which

cardiac malformations were observed in fetuses following the oral administration of TCE to maternal animals during gestation (Johnson et al., 2005, 2003; Dawson et al., 1993, 1990) (see Section 4.8.3.2.1.2). Cardiac defects were also observed in rats following oral gestational treatment with metabolites of TCE (Johnson et al., 1998b; Johnson et al., 1998a; Epstein et al., 1992; Smith et al., 1992; Smith et al., 1989).

However, cardiac malformations were not observed in a number of other studies in laboratory animals in which TCE was administered during the period of cardiac organogenesis and fetal visceral findings were assessed. These included inhalation studies in rats (Carney et al., 2006; Healy et al., 1982; Hardin et al., 1981; Dorfmueller et al., 1979; Schwetz et al., 1975) and rabbits (Hardin et al., 1981), and gavage studies in rats (Fisher et al., 2001; Narotsky and Kavlock, 1995; Narotsky et al., 1995) and mice (Cosby and Dukelow, 1992).

It is generally recognized that response variability among developmental bioassays conducted with the same chemical agent may be related to factors such as the study design (e.g., the species and strain of laboratory animal model used, the day(s) or time of day of dose administration in relation to critical developmental windows, the route of exposure, the vehicle used, the day of study termination), or the study methodologies (e.g., how fetuses were processed, fixed, and examined; what standard procedures were used in the evaluation of morphological landmarks or anomalies; and whether there was consistency in the fetal evaluations that were conducted). In the case of studies that addressed cardiac malformations, there is additional concern as to whether detailed visceral observations were conducted and whether or not cardiac evaluation was conducted using standardized dissection procedures (e.g., with the use of a dissection microscope or including confirmation by histopathological evaluation, and whether the examinations were conducted by technicians who were trained and familiar with fetal cardiac anatomy). Furthermore, interpretation of the findings can be influenced by the analytical approaches applied to the data as well as by biological considerations such as the historical incidence data for the species and strain of interest. These issues have been critically examined in the case of the TCE developmental toxicity studies (Watson et al., 2006; Hardin et al., 2005).

In the available animal developmental studies with TCE, differences were noted in the procedures used to evaluate fetal cardiac morphology following TCE gestational exposures across studies, and some of these differences may have resulted in inconsistent fetal outcomes and/or the inability to detect cardiac malformations. Most of the studies that did not identify cardiac anomalies used a traditional free-hand sectioning technique (as described in Wilson, 1965) on fixed fetal specimens (Healy et al., 1982; Hardin et al., 1981; Dorfmueller et al., 1979; Schwetz et al., 1975). Detection of cardiac anomalies can be enhanced through the use of a fresh dissection technique as described by Staples (1974) and Stuckhardt and Poppe (1984); a significant increase in treatment-related cardiac heart defects was observed by Dawson et al. (1990) when this technique was used. Further refinement of this fresh dissection technique was

employed by Dawson and colleagues at the University of Arizona (UA), resulting in several additional studies that reported cardiac malformations (Johnson et al., 2005, 2003; Dawson et al., 1993). However, two studies conducted in an attempt to verify the teratogenic outcomes of the UA laboratory studies used the same or similar enhanced fresh dissection techniques and were unable to detect cardiac anomalies (Carney et al., 2006; Fisher et al., 2001). Although the Carney et al. (2006) study was administered via inhalation (a route that has not previously been shown to produce positive outcomes), the Fisher et al. (2001) study was administered orally and included collaboration between industry and UA scientists. It was suggested that the apparent differences between the results of the Fisher et al. (2001) study and the Dawson et al. (1993) and Johnson et al. (2003) studies may be related to factors such as differences in purity of test substances or in the rat strains, or differences in experimental design (e.g., gavage vs. drinking water, exposure only during the period of organogenesis versus during the entire gestation period, or the use of a staining procedure).

It is notable that all studies that identified cardiac anomalies following gestational exposure to TCE or its metabolites were: (1) conducted in rats and (2) dosed by an oral route of exposure (gavage or drinking water). Cross-species and route-specific differences in fetal response may be due in part to toxicokinetic factors. Although a strong accumulation and retention of TCA was found in the amniotic fluid of pregnant mice following inhalation exposures to TCE (Ghantous et al., 1986), other toxicokinetic factors may be critical. The consideration of toxicokinetics in determining the relevance of murine developmental data for human risk assessment is briefly discussed by Watson et al. (2006). There are differences in the metabolism of TCE between rodent and humans in that TCE is metabolized more efficiently in rats and mice than humans, and a greater proportion of TCE is metabolized to DCA in rodents versus to TCA in humans. Studies that examined the induction of cardiac malformations with gestational exposures of rodents to various metabolites of TCE identified TCA and DCA as putative cardiac teratogens. Johnson et al. (1998b; 1998a) and Smith et al. (1989) reported increased incidences of cardiac defects with gestational TCA exposures, while Smith et al. (1992) and Epstein et al. (1992) reported increased incidences following DCA exposures.

In all studies that observed increased cardiac defects, either TCE or its metabolites were administered during critical windows of in utero cardiac development, primarily during the entire duration of gestation, or during the period of major organogenesis (e.g., GDs 6–15 in the rat). The study by Epstein et al. (1992) used dosing with DCA on discrete days of gestation and had identified GDs 9 through 12 as a particularly sensitive period for eliciting high interventricular septal defects associated with exposures to TCE or its metabolites.

In the oral studies that identified increased incidences of cardiac malformations following gestational exposure to TCE, there was a broad range of administered doses at which effects were observed. In drinking water studies, Dawson et al. (1993) observed cardiac anomalies at 1.5 and 1,100 ppm (with no NOAEL) and Johnson et al. (2005, 2003) reported effects at 250 ppb

(with a NOAEL of 2.5 ppb). One concern is the lack of a clear dose-response for the incidence of any specific cardiac anomaly or combination of anomalies was not identified, a disparity for which no reasonable explanation for this disparity has been put forth.

The analysis of the incidence data for cardiac defects observed in the Dawson et al. (1993, 1990) and Johnson et al. (2005, 2003) studies has been critiqued (Watson et al., 2006). Issues of concern that have been raised include the statistical analyses of findings on a per-fetus (rather than the more appropriate per-litter) basis (Benson, 2004). Johnson et al. was further criticized for the use of nonconcurrent control data in the analysis (Hardin et al., 2004). In response, the study author has further explained procedures used (Johnson et al., 2004) and has provided individual litter incidence data to the EPA for independent statistical analysis (P. Johnson, personal communication, 2008) (see Section 5.1.2.8). In sum, while the studies by Dawson et al. (1993, 1990) and Johnson et al. (2005, 2003), have significant limitations, there is insufficient reason to dismiss their findings.

4.8.3.3.2.1. Mode of action for cardiac malformations

A number of in vitro studies have been conducted to further characterize the potential for alterations in cardiac development that have been attributed to exposures with TCE and/or its metabolites. It was noted that many of the cardiac defects observed in humans and laboratory species (primarily rats and chickens) involved septal and valvular structures.

During early cardiac morphogenesis, outflow tract and AV endothelial cells differentiate into mesenchymal cells. These mesenchymal cells have characteristics of smooth muscle-like myofibroblasts and form endocardial cushion tissue, which is the primordia of septa and valves in the adult heart. Events that take place in cardiac valve formation in mammals and birds are summarized by NRC (2006) and reproduced in Table 4-104.

Table 4-104. Events in cardiac valve formation in mammals and birds[a]

Stage and event	Structural description[b]
Early cardiac development	The heart is a hollow, linear, tube-like structure with two cell layers. The outer surface is a myocardial cell layer, and the inner luminal surface is an endothial layer. Extracellular matrix is between the two cell layers.
Epithelial-mesenchymal cell transformation	A subpopulation of endothelial cells lining the AVC detaches from adjacent cells and invades the underlying extracellular matrix. Three events occur: ➢ Endothelial cell activation (avian stage 14) ➢ Mesenchymal cell formation (avian stage 16) ➢ Mesenchymal cell migration into the extracellular matrix (avian stages 17 and 18).
Mesenchymal cell migration and proliferation	Endothelial-derived mesenchymal cells migrate toward the surrounding myocardium and proliferate to populate the AVC extracellular matrix.
Development of septa and valvular structures	Cardiac mesenchyme provides cellular constituents for: ➢ Septum intermedium ➢ Valvular leaflets of the mitral and tricuspid AV valves. The septum intermedium subsequently contributes to: ➢ Lower portion of the interatrial septum ➢ Membranous portion of the interventricular septum.

[a]As summarized in NRC (2006).
[b]Markwald et al. (1996; 1984); Boyer et al. (2000).

Methods have been developed to extract the chick stage 16 AVC from the embryo and culture it on a hydrated collagen gel for 24–48 hours, allowing evaluation of the described stages of cardiac development and their response to chemical treatment. Factors that have been shown to influence the induction of endocardial cushion tissue include molecular components such as fibronectin, laminin, and galactosyltransferase (Loeber and Runyan, 1990; Mjaatvedt et al., 1987), components of the extracellular matrix (Mjaatvedt et al., 1991), and smooth muscle α-actin and transforming growth factor β3 (Nakajima et al., 1997; Ramsdell and Markwald, 1997).

Boyer et al. (2000) utilized the in vitro chick AVC culture system to examine the molecular mechanism of TCE effects on cardiac morphogenesis. AVC explants from stage 16 chick embryos (15/treatment level) were placed onto collagen gels and treated with 0, 50, 100, 150, 200, or 250 ppm TCE and incubated for a total of 54 hours. Epithelial-mesenchymal transformation, endothelial cell density, cell migration, and immunohistochemistry were evaluated. TCE treatment was found to inhibit endothelial cell activation and normal mesenchymal cell transformation, endothelial cell-cell separation, and protein marker expression (i.e., transcription factor Mox-1 and extracellular matrix protein fibrillin 2). Mesenchymal cell migration was not affected, nor was the expression of smooth muscle α-actin. The study authors proposed that TCE may cause cardiac valvular and septal malformations by inhibiting endothelial separation and early events of mesenchymal cell formation. Hoffman et al. (2004) proposed alternatively that TCE may be affecting the adhesive properties of the endocardial cells. No experimental data are currently available that address the levels of TCE in cardiac

tissue in vivo, resulting in some questions (Dugard, 2000) regarding the relevance of these mechanistic findings to human health risk assessment.

In a study by Mishima et al. (2006), White Leghorn chick whole embryo cultures (stage 13 and 14) were used to assess the susceptibility of endocardial epithelial-mesenchymal transformation in the early chick heart to TCE at analytically determined concentrations of 0, 10, 20, 40, or 80 ppm. This methodology maintained the anatomical relationships of developing tissues and organs, while exposing precisely staged embryos to quantifiable levels of TCE and facilitating direct monitoring of developmental morphology. Following 24 hours of incubation, the numbers of mesenchymal cells in the inferior and superior AV cushions were counted. TCE treatment significantly reduced the number of mesenchymal cells in both the superior and inferior AV cushions at 80 ppm.

Ou et al. (2003) examined the possible role of endothelial nitric oxide synthase (which generates nitric oxide that has an important role in normal endothelial cell proliferation and hence normal blood vessel growth and development) in TCE-mediated toxicity. Cultured proliferating bovine coronary endothelial cells were treated with TCE at 0–100 μM and stimulated with a calcium ionophore to determined changes in endothelial cells and the generation of endothelial nitric oxide synthase, nitric oxide, and superoxide anion. TCE was shown to alter heat shock protein interactions with endothelial nitric oxide synthase and induce endothelial nitric oxide synthase to shift nitric oxide to superoxide-anion generation. These findings provide insight into how TCE impairs endothelial proliferation.

Several studies have also identified a TCE-related perturbation of several proteins involved in regulation of intracellular Ca^{2+}. After 12 days of maternal exposure to TCE in drinking water, *Serca2a* (sarcoendoplasmic reticulum Ca^{2+} ATPase) mRNA expression was reduced in rat embryo cardiac tissues (Collier et al., 2003). Selmin et al. (2008) conducted a microarray analysis of a P19 mouse stem cell line exposed to 1-ppm TCE in vitro, identifying altered expression of *Ryr2* (ryanodine receptor isoform 2), a Ca^{2+} release channel that is important in normal rhythmic heart activity (Gyorke and Terentyev, 2008). Alterations in Ca^{2+} cycling and resulting contractile dysfunction is a recognized pathogenic mechanism of cardiac arrhythmias and sudden cardiac death (Lehnart et al., 2008; Yano et al., 2008; Leandri et al., 1995). Caldwell et al. (2008c) used real-time PCR and digital imaging microscopy to characterize the effects of various doses of TCE on gene expression and Ca^{2+} response to vasopressin in rat cardiac myocytes (H9c2) *Serca2a* and *Ryr2* expression were reduced at 12 and 48 hours following exposure to TCE. Additionally, Ca^{2+} response to vasopressin was altered following TCE treatment. Makwana et al. (2010) dosed chick embryos in ovo with 8 or 800 ppb TCE; real time-PCR analysis of RNA isolated during specific windows of cardiac development demonstrated effects on the expression of genes associated with reduced blood flow. Although it has been hypothesized that TCE might interfere with the folic acid/methylation pathway in liver and kidney and alter gene regulation by epigenetic

mechanisms, Caldwell et al. (2010) found that the effects of TCE exposure on normal gene expression in rat embryonic hearts was not altered by the administration of exogenous folate. Overall, these data suggest that TCE may disrupt the ability to regulate cellular Ca^{2+} fluxes, altering blood flow and leading to morphogenic consequences in the developing heart. This remains an open area of research.

Thus, in summary, a number of studies have been conducted in an attempt to characterize the mode of action for TCE-induced cardiac defects. A major research focus has been on disruptions in cardiac valve formation, using avian in ovo and in vitro studies. These studies demonstrated treatment-related alterations in endothelial cushion development that could plausibly be associated with defects involving septal and valvular morphogenesis in rodents and chickens. However, a broad array of cardiac malformations has been observed in animal models following TCE exposures (Johnson et al., 2005, 2003; Dawson et al., 1993), and other evidence of molecular disruption of Ca^{2+} during cardiac development has been examined (Caldwell et al., 2008c; Selmin et al., 2008; Collier et al., 2003), suggesting the possible existence of multiple modes of action. The observation of defective myocardial development in a mouse model deficient for gp130, a signal transducer receptor for IL-6 (Yoshida et al., 1996), suggests the potential involvement of immune-mediated effects.

4.8.3.3.2.2. Association of PPARα with developmental outcomes

The PPARs are ligand activated receptors that belong to the nuclear hormone receptor family. Three isotypes have been identified (PPARα, PPARδ [also known as PPARβ], and PPARγ). These receptors, upon binding to an activator, stimulate the expression of target genes implicated in important metabolic pathways. In rodents, all three isotypes show specific time- and tissue-dependent patterns of expression during fetal development and in adult animals. In development, they have been especially implicated in several aspects of tissue differentiation (e.g., of the adipose tissue, brain, placenta, and skin). Epidermal differentiation has been linked strongly with PPARα and PPARδ (Michalik et al., 2002). PPARα starts late in development, with increasing levels in organs such as liver, kidney, intestine, and pancreas; it is also transiently expressed in fetal epidermis and CNS (Braissant and Wahli, 1998) and has been linked to phthalate-induced developmental and testicular toxicity (Corton and Lapinskas, 2005). Liver, kidney, and heart are the sites of highest PPARα expression (Toth et al., 2007). PPARδ and PPARγ have been linked to placental development and function, with PPARγ found to be crucial for vascularization of the chorioallantoic placenta in rodents (Wendling et al., 1999), and placental anomalies mediated by PPARγ have been linked to rodent cardiac defects (Barak et al., 2008). While it might be hypothesized that there is some correlation between PPAR signaling, fetal deaths, and/or cardiac defects observed following TCE exposures in rodents, no definitive data have been generated that elucidate a possible PPAR-mediated mode of action for these outcomes.

4.8.3.3.2.3. Summary of the weight of evidence on cardiac malformations

The evidence for an association between TCE exposures in the human population and the occurrence of congenital cardiac defects is not particularly strong. Many of the epidemiological study designs were not sufficiently robust to detect exposure-related birth defects with a high degree of confidence. However, two well-conducted studies by ATSDR (2008b, 2006a) clearly demonstrated an elevation in cardiac defects. It could be surmised that the identified cardiac defects were detected because they were severe, and that additional cases with less severe cardiac anomalies may have gone undetected.

The animal data provide strong, but not unequivocal, evidence of the potential for TCE-induced cardiac malformations following oral exposures during gestation. Strengths of the evidence are the duplication of the adverse response in several studies from the same laboratory group, detection of treatment-related cardiac defects in both mammalian and avian species (i.e., rat and chicken), general cross-study consistency in the positive association of increased cardiac malformations with test species (i.e., rat), route of administration (i.e., oral), and the methodologies used in cardiac morphological evaluation (i.e., fresh dissection of fetal hearts). Furthermore, when differences in response are observed across studies, they can generally be attributed to obvious methodological differences, and a number of in ovo and in vitro studies demonstrate a consistent and biologically plausible mode of action for one type of malformation observed. Weaknesses in the evidence include lack of a clear dose-related response in the incidence of cardiac defects, and the broad variety of cardiac defects observed, such that they cannot all be grouped easily by type or etiology.

Taken together, the epidemiological and animal study evidence raise sufficient concern regarding the potential for developmental toxicity (increased incidence of cardiac defects) with in utero TCE exposures.

4.8.3.3.3. Other structural developmental outcomes

A summary of other structural developmental outcomes that have been associated with TCE exposures is presented in Table 4-105.

Table 4-105. Summary of other structural developmental outcomes associated with TCE exposures

Finding	Species	References
Eye/ear birth anomalies	Human	Lagakos et al. (1986)
	Rat	Narotsky (1995); Narotsky and Kavlock (1995)
Oral cleft defects	Human	Bove (1996); Bove et al. (1995); Lagakos et al. (1986); Lorente et al. (2000)
Kidney/urinary tract disorders	Human	Lagakos et al. (1986)
Musculoskeletal birth anomalies	Human	Lagakos et al. (1986)
Anemia/blood disorders	Human	Burg and Gist (1999)
Lung/respiratory tract disorders	Human	Lagakos et al. (1986)
	Mouse	Das and Scott (1994)
Skeletal	Rat	Healy et al. (1982)
Other[a]	Human	ATSDR (2001)

[a]As reported by the authors.

In humans, a variety of birth defects other than cardiac have been observed. These include total birth defects (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Flood, 1988) CNS birth defects (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986), eye/ear birth anomalies (Lagakos et al., 1986); oral cleft defects (Lorente et al., 2000; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986); kidney/urinary tract disorders (Lagakos et al., 1986); musculoskeletal birth anomalies (Lagakos et al., 1986); anemia/blood disorders (Burg and Gist, 1999); and lung/respiratory tract disorders (Lagakos et al., 1986). While some of these results were statistically significant, they have not been reported elsewhere. Occupational cohort studies, while not reporting positive results, are generally limited by the small number of observed or expected cases of birth defects (Lorente et al., 2000; Taskinen et al., 1989; Tola et al., 1980).

In experimental animals, a statistically significant increase in the incidence of fetal eye defects, primarily micropththalmia and anopththalmia, manifested as reduced or absent eye bulge, was observed in rats following gavage administration of 1,125 mg/kg-day TCE during the period of organogenesis (Narotsky and Kavlock, 1995; Narotsky et al., 1995). Dose-related nonsignificant increases in the incidence of F344 rat pups with eye defects were also observed at lower dose levels (101, 320, 475, 633, and 844 mg/kg-day) in the Narotsky et al. (1995) study (also reported in Barton and Das, 1996). However, no other developmental or reproductive toxicity studies identified abnormalities of eye development following TCE exposures. For example, in a study reported by Warren et al. (2006), extensive computerized morphometric ocular evaluation was conducted in Sprague-Dawley rat fetuses that had been examined for cardiac defects by Fisher et al. (2001); the dams had been administered TCE (500 mg/kg-day),

DCA (300 mg/kg-day), or TCA (300 mg/kg-day) during GDs 6–15. No ocular defects were found with TCE exposures; however, significant reductions in the lens area, globe area, and interocular distance were observed with DCA exposures, and nonsignificant decreases in these measures as well as the medial canthus distance were noted with TCA exposures. Developmental toxicity studies conducted by Smith et al. (1992; 1989) also identified orbital defects (combined soft tissue and skeletal abnormalities) in Long-Evans rat fetuses following GD 6–15 exposures with TCA and DCA (statistically or biologically significant at ≥800 and ≥900 mg/kg-day, respectively). Overall, the study evidence indicates that TCE and its oxidative metabolites can disrupt ocular development in rats. In addition to the evidence of alteration to the normal development of ocular structure, these findings may also be an indicator of disruptions to nervous system development. It has been suggested by Warren et al. (2006) and Williams and DeSesso (2008) that the effects of concern (defined as statistically significant outcomes) are observed only at high dose levels and are not relevant to risk assessment for environmental exposures. On the other hand, Barton and Das (1996) point out that BMD modeling of the quantal eye defect incidence data provides a reasonable approach to the development of oral toxicity values for TCE human health risk assessment. It is also noted that concerns may exist not only for risks related to low level environmental exposures, but also for risks resulting from acute or short-term occupational or accidental exposures, which may be associated with much higher inadvertent doses.

It was also notable that a study using a single i.p. dose of 3,000 mg/kg TCE to mice during late gestation (GD 17) identified apparent delays in lung development and increased neonatal mortality (Das and Scott, 1994). No further evaluation of this outcome has been identified in the literature.

Healy et al. (1982) did not identify any treatment-related fetal malformations following inhalation exposure of pregnant inbred Wistar rats to 0 or 100 ppm (535 mg/m^3) on GDs 8–21. In this study, significant differences between control and treated litters were observed as an increased incidence of minor ossification variations ($p = 0.003$) (absent or bipartite centers of ossification).

4.8.3.3.4. Developmental neurotoxicity

Studies that address effects of TCE on the developing nervous system are discussed in detail in Section 4.3, addressed above in the sections on human developmental toxicity (see Section 4.8.3) and on mammalian studies (see Section 4.8.3.2.1) by route of exposure, and summarized in Table 4-106. The available data collectively suggest that the developing brain is susceptible to TCE exposures.